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DIFFERENCES IN THE DURATION OF EGG DIAPAUSE OF *LABIDOCERA AESTIVA* (COPEPODA: CALANOIDA) FROM THE WOODS HOLE, MASSACHUSETTS, REGION

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Abstract

The duration of diapause of *Labidocera aestiva* eggs collected from the field and reared in the laboratory was determined at 5°C. A clear seasonal trend was observed. Diapause eggs produced in the early fall required a much longer exposure to cold to yield a 50% hatch (CT_{50}) (*i.e.*, the duration of diapause was longer) than eggs produced later in the fall. Eggs produced by laboratory animals that were reared at 14°C, 8L–16D, required a shorter period of chilling to terminate diapause than the eggs of animals reared at 19°C, 12L–12D. Considerable variation in the CT_{50} value was also observed among laboratory cultures that were all reared under identical conditions, but which differed in terms of selection history. The results indicate that both the genotype of the egg and the conditions prevailing during oocyte formation influence the duration of diapause. Eggs that were stored at 5°C for periods longer than 300 days no longer hatched upon warming. It is suggested that the variation in the duration of diapause is an adaptation that promotes synchronization of hatching by ensuring that all individuals terminate diapause at approximately the same time, and survival during the winter by conferring cold-hardiness. Synchronizing the onset of post-diapause development is also discussed as an alternative mechanism for achieving synchronous hatching.

Introduction

The calanoid copepod, *Labidocera aestiva,* is a seasonal member (summer and fall) of the planktonic community in the Woods Hole region. In this area most *L. aestiva* females have the genetic potential to produce two types of eggs: subitaneous and diapause (Marcus, 1982). Subitaneous eggs are produced during the summer and fall; diapause eggs are produced during the fall. Both egg types begin to develop following their release by females. Subitaneous eggs typically hatch within 1 to 4 days at 21 to 23°C (Marcus, 1979). Diapause eggs enter a refractory phase after 24 to 48 h of development. During the refractory phase, further embryogenesis is not apparent (Marcus, pers. obs.) and diapause eggs cannot be induced to hatch even if conditions are favorable. The duration of diapause (*i.e.*, the length of the refractory period) is positively related to the temperature at which eggs are held (Grice and Gibson, 1975; Marcus, 1979). Once the refractory phase is completed, post-diapause development and hatching occurs if conditions (*e.g.*, temperature) are favorable. For instance, at 21 to 23°C hatching typically occurs within 1 to 2 days (Marcus, 1979). Several field and laboratory studies (Marcus, 1979, 1980, 1984) support the claim that the perpet-

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uation of *L. aestiva* year after year in the Woods Hole region is due to the diapause eggs which overwinter on the sea-bottom and hatch in the spring.

During field and laboratory studies on *L. aestiva* I observed that many diapause eggs could be induced to hatch at 19°C following a chilling period of 4 weeks at 5°C. However, some eggs would hatch with a shorter period of chilling while others required even longer exposure to cold. A comparison of diapause eggs obtained from females collected in the field showed that the period of chilling that would result in a 50% hatch at 19°C was longer for the diapause eggs of females collected early in the fall (Marcus, 1986). This study examines in more detail the seasonal variation in the duration of diapause of eggs of freshly caught animals from the field and compares the results to values obtained for diapause eggs of females reared in the laboratory. The results indicate that the genotype of an egg and the environmental factors acting during oocyte formation influence the duration of diapause. Based on the results, I suggest that the variation in the duration of diapause is an adaptation that promotes synchronization of hatching in the field by ensuring that all individuals terminate diapause at approximately the same time, and survival during the winter by conferring cold-hardiness.

Materials and Methods

Diapause eggs were obtained from animals collected at one to two week intervals over a period of 2 years from October 1981 to October 1983. For each sampling date, adult females were collected from Vineyard Sound by towing a ¾ m diameter, 243 μ mesh plankton net for 10 min. Water temperature was determined on a surficial bucket sample for all but two collection dates. For these dates, water temperature was estimated based on the daily temperature record for water off the Woods Hole Oceanographic Institution dock. A comparison of several dates showed that the WHOI values were typically about 1°C less than Vineyard Sound values. Field sampling dates and surface water temperature at the time of collection are shown in Table I. In the laboratory females were transferred to 100 ml dishes containing 5 μm-filtered seawater and the dinoflagellate *Gymnodinium nelsoni* (500 cells/ml). The dishes were incubated overnight at 19°C. The next day eggs were collected by pipette, pooled in a separate dish of filtered seawater, and returned to the incubator for 2–4 days to allow the subitaneous eggs to hatch. Unhatched eggs that appeared to be diapause eggs (*i.e.*, the interiors were green, with a clear perimeter) were distributed into 75 ml glass screw capped jars (20–25 eggs/jar) containing filtered seawater, and refrigerated at 5°C. Eggs that were obviously non-viable (*i.e.*, the interiors were brownish, granular, and disintegrating) were discarded. Every 2 to 4 days, a jar was removed (except for the 2 collections in 1981 for which duplicate jars were removed), warmed to 19°C, and held at that temperature. After 4 to 5 days the proportion of hatched eggs was ascertained. The average hatch of the duplicates was recorded for the two 1981 collection dates.

Eggs of laboratory-reared animals were from 12 different cultures that were reared either at a temperature of 14 or 19°C (±1°C), and a photoperiodic regimen of 8L–16D or 12L–12D. The eggs collected from each culture were 1–2 days old. The adults were approximately 2 weeks past reproductive maturity. The cultures represented specific generations of three inbred lines that were being perpetuated as part of a long-term selection experiment designed to assess the potential for evolutionary change in the diapause response threshold (*i.e.*, the necessary conditions for the expression of diapause). Each line was initiated from 500–1000 nauplii that were derived from pooled batches of either subitaneous or diapause eggs produced by 60 females col-

TABLE I

Collection dates, surface water temperature (°C), chilling time (days) required for initial hatch, CT_{50} values (days), and regression parameters pertaining to diapause eggs of field collected females

Date	°C	Initial	CT_{50}	r^2	Slope
9/20/82	19	14	23.19	.88	7.31
9/26/83	21	18	28.82	.80	9.07
9/27/82	19	12	28.61	.79	4.14
10/04/82	18	14	27.40	.88	6.03
10/11/83	18	23	26.13	.90	13.69
10/12/82	17	16	23.02	.78	10.11
10/19/81	14	4	15.44	.89	3.66
10/21/82	14	6	24.14	.74	3.71
10/24/83	16	20	22.37	.84	8.61
11/01/82	14	8	17.30	.91	5.25
11/10/82	13	6	16.53	.90	4.81
11/23/82	11	4	15.36	.80	3.22
11/29/82	10	2	8.98	.89	3.44
12/01/81	7	6	10.99	.99	3.16
12/08/82	10	2	10.17	.96	2.71

lected from the field. Two of the lines were termed subitaneous. Each generation was perpetuated from 500–1000 nauplii derived from just the subitaneous eggs that were produced by the preceding generation of animals. A third diapause line was perpetuated in a similar manner, but from just diapause eggs. The specific rearing conditions and selection histories are shown in Table II. The proportion of subitaneous and diapause eggs produced by each generation of animals varied within and between the lines. The diet for all cultures consisted of a standard mix of four dinoflagellates. General methods for rearing of *L. aestiva* have been described previously (Marcus, 1980). Eggs from each culture were incubated at 19°C for 4 to 5 days after which the diapause eggs were distributed (20 to 30 eggs/jar) into 75 ml jars. The jars were refrigerated at 5°C. At 2 to 4 day intervals the jars were removed, warmed to 19°C, and held at that temperture. The proportion of eggs that hatched after 4 to 5 days was determined.

For each field sampling date and laboratory culture, values of percent hatch were transformed to probit values (Finney, 1952). A regression analysis was performed with these values *versus* the number of days chilled ($\log_{10}$) to derive an estimate of the days of chilling required to promote a 50% hatch (CT_{50}). Calculations were done with an IBM PC and the statistical software package, STATPRO.

The effect of long-term storage at 5°C on egg viability and hatching was examined for the 2 sets of diapause eggs obtained from culture 339. After the initial analysis period, jars of eggs were removed at intervals of up to 4 weeks for more than a year. The hatch of these eggs after warming to 19°C was ascertained as described above.

RESULTS

In general, a shorter period of chilling was necessary to promote initial and 50% hatching of eggs produced by females collected from the field later in the fall (Table I, Fig. 1). The results of the Probit transformation and regression analysis permit a quantified comparison of these differences and the derivation of the median effective chilling period (*i.e.*, the number of days of chilling that promote a 50% hatch). The

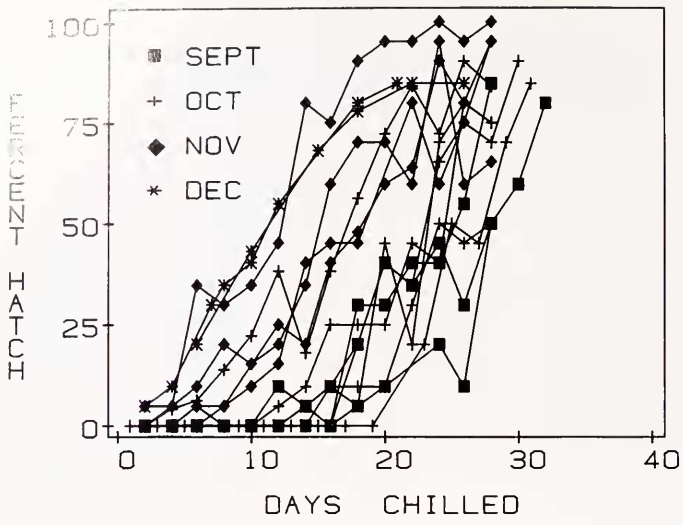

FIGURE 1. Percent hatch of diapause eggs, from field-collected females, at 19°C after chilling at 5°C for the designated number of days. Each set of connected points represents a specific sampling date. Dates for each month are grouped by the indicated symbols.

regression parameters (slope, r^2) and CT_{50} values are shown in Table I. The coefficient of determination values (r^2) ranged from .74 to .99 indicating that the linear regression relationship was a good one for estimating the CT_{50}. For the diapause eggs of field-collected females, the CT_{50} values ranged from 8.98 to 28.82 days. The slope values of the regression ranged from 2.71 to 13.69 probit value/days (log_{10}). This latter parameter provides an indication of the time spread of diapause duration around the median. A high value corresponds to a very short interval for the time from initial to maximal hatching. The highest values tended to occur during September and October, and the lowest during November and December. This same pattern was found for the CT_{50} values. Further analysis revealed that a very good positive correlation ($r^2 = .84$) existed between CT_{50} values and surface water temperature at the time of sampling (Fig. 2).

The median effective duration of chilling also differed among the laboratory reared groups although the range of values was not as great as observed for the field group. The regression parameters (slope, r^2) and CT_{50} values are shown in Table II. As for the field group the r^2 values were high (.71 to .94). The CT_{50} values ranged from 5.82 to 21.09 days. The slope values of the regression ranged from 1.70 to 5.23 probit value/days (log_{10}). The lowest CT_{50} values were obtained for the 3 cultures (370, 371, 374) that were reared at 14°C and 8L–16D. For 2 pairs of cultures, 370 and 372, and 371 and 373, the within pair cultures were established from the same pool of eggs in the 23rd generation, but were reared at the two alternative sets of conditions. In both cases the CT_{50} values were lower for the cultures reared at 14°C, and 8L–16D. A third unpaired culture (374) was reared at 14°C and 8L–16D and also yielded the third lowest CT_{50} value.

The two sets of eggs that were obtained from culture 339 were collected on different days and the CT_{50} values differed by almost 5 days. The long term response to chilling was also different for the two sets (Fig. 3). The hatch after chilling increased more rapidly during the first 30 days for 339b, but a high hatch was maintained for

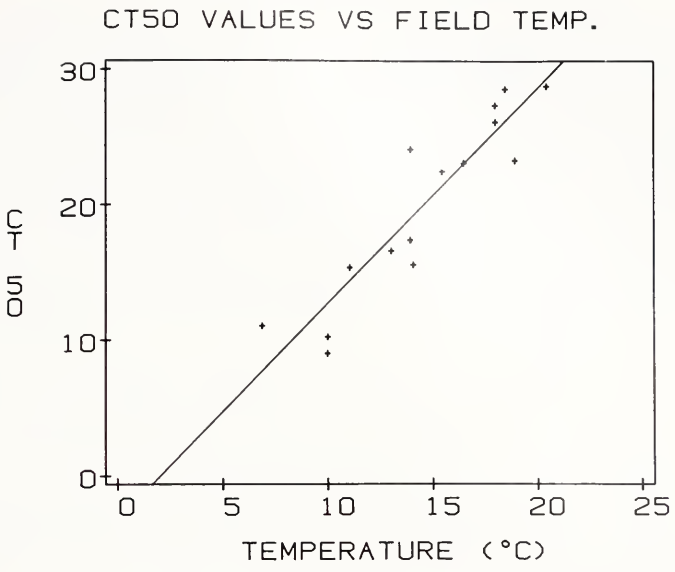

FIGURE 2. Linear regression analysis of CT_{50} value of each field sample and the surface water temperature at the time of collection.

only 150 to 200 days whereas a high hatch was maintained by the eggs of 339a for almost 300 days. Although the hatch of both sets dropped off to near 0% levels after 300 days, many of the eggs in both sets still appeared viable.

DISCUSSION

This study shows that the median effective number of days of chilling at 5°C decreased as the fall season progressed for the diapause eggs of field-collected females.

TABLE II

Culture # (generation, subitaneous-s or diapause-d line), rearing conditions (photoperiod and temperature), CT_{50} values (days), and regression parameters pertaining to diapause eggs of laboratory-reared females

Culture #	Conditions	CT_{50}	r^2	Slope
370 (23s)	14°C, 8L–16D	10.43	.85	2.18
371 (23s)	14°C, 8L–16D	5.82	.71	1.70
374 (6s)	14°C, 8L–16D	10.74	.90	2.92
375 (3s)	19°C, 12L–12D	12.16	.91	2.59
372 (23s)	19°C, 12L–12D	11.77	.78	3.11
373 (23s)	19°C, 12L–12D	14.33	.74	2.37
376 (7d)	19°C, 12L–12D	19.33	.78	2.41
323 (7s)	19°C, 12L–12D	17.92	.83	4.42
329 (15s)	19°C, 12L–12D	13.64	.87	3.04
325 (4d)	19°C, 12L–12D	21.09	.78	3.27
339a(5d)*	19°C, 12L–12D	16.59	.93	5.23
339b(5d)*	19°C, 12L–12D	11.81	.94	3.73
330 (15s)	19°C, 8L–16D	15.66	.84	3.54

* Eggs collected from same culture, but on two different days.

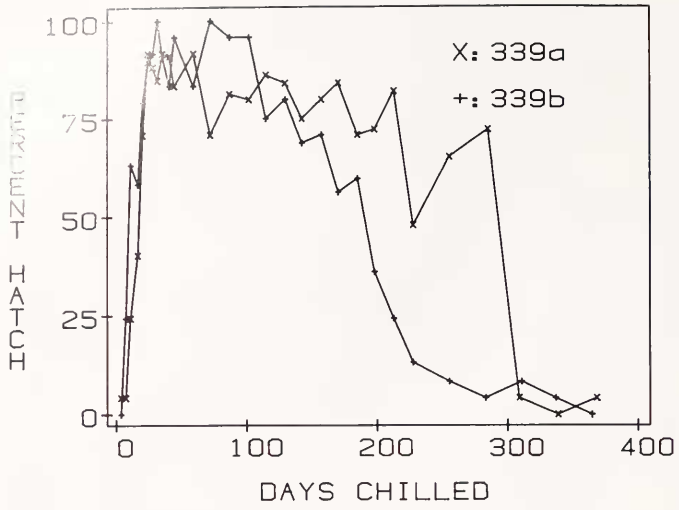

FIGURE 3. Percent hatch of diapause eggs, produced by culture 339 on different days (a and b), at 19°C after chilling at 5°C for the designated number of days.

The range of values spanned 20 days. Similar seasonal trends have been reported for the diapause stages of insects (Burdick, 1937; Church and Salt, 1952). During the collecting period of *L. aestiva,* water temperatures ranged from 20.5 to 6.9°C, a difference of approximately 14.0°C. The two temperatures at which the laboratory animals were reared differed by 5°C and the number of days of chilling required to achieve a 50% hatch differed by as much as 15.5 days. At a constant 19°C, the range in CT_{50} values was about 10 days for the eggs of laboratory-reared animals. Thus for the laboratory-reared animals considerable variation in the median effective days of chilling was obtained, despite the fact that the environmental conditions were the same. This variation must reflect genetic differences. Further evidence for genetic variation are the different responses observed for the eggs of culture 339 that were collected on different days. Since the eggs all came from the same culture, the only possible explanation is that the eggs collected on the different days were produced by different mixes of parents. Although the cultures of animals that were used for the analyses represented different generations of three genetically distinct lines, no obvious association was observed between diapause duration and generation number or selection history.

Although genetic differences appear to be important, environmental factors may also have an effect. The three shortest times to achieve a 50% hatch were obtained for the cultures that had been reared at 14°C and 8L–16D (Table II). This same relationship with temperature and short-daylengths was evident for the eggs of the field collected animals. The work of Denlinger and Bradfield (1981) on the tobacco hornworm provides a possible explanation for these trends. They showed that the duration of diapause was influenced by the number of short day cycles perceived by individuals. As the number of short day cycles experienced by an individual increased, the duration of diapause decreased. They concluded that in the field the duration of diapause is shorter for individuals entering diapause late in the fall because declining temperatures lead to slower development and therefore a longer exposure to short daylengths. If this mechanism characterizes *L. aestiva* it is unlikely that the oocyte

itself could perceive the number of short day cycles. Hence, the effect would have to be mediated through the parent female as a "maternal effect." In the case of *L. aestiva,* it is also possible that declining temperatures directly influence the physiological state of the female and, in turn, oogenesis. The differences observed for the eggs of field-collected animals could also result from variation in maternal age. Animals collected late in the fall might be older than ones collected early in the fall. However, this would not explain the variation expressed by the eggs of laboratory-reared females since all of the animals were similar in age. Krysan and Branson (1977) conducted specific crosses with the corn rootworm and showed that the duration of diapause was affected both by the genotype of the embryo and a maternal component. Further experiments are needed to assess the relative importance of these components in *L. aestiva.*

The long term response to chilling observed for the eggs of culture 339 (Fig. 3) is very similar to patterns observed for several insect species (Hussey, 1955; Cranham, 1972; Lees, 1955). For these species, the percent of individuals terminating diapause increased to a maximum with increasing length of exposure to cold, then remained high with longer exposure to cold, and finally declined with excessive time of exposure to cold. In each case the percent terminating diapause remained low after excessive exposure, but the interpretation of the results differed among authors. Hussey (1955) suggested that the decline was part of an annual cycle and that given enough time the percent terminating diapause would increase again. Hussey believed that there was an internal gating rhythm that controlled emergence from diapause. However, since he did not carry the experiments through for another year it cannot be certain that death had not occurred. The rhythm concept was not discussed by the others despite similar results. They concluded that the eggs had lost viability and would never hatch. It would certainly be advantageous for an egg of *L. aestiva* to remain viable beyond one season. *L. aestiva* eggs that are buried do not hatch despite favorable temperatures (Marcus and Schmidt-Gengenbach, 1986). The probability of completing development should be higher for a diapause egg (from the previous fall) that is uncovered no later than the summer than for an egg which is not uncovered until October or November. If hatching occurred only in October and November, the likelihood of completing development should be diminished due to declining temperatures. A gating rhythm that controlled the onset of post-diapause development would reduce the probability of eggs hatching at an inappropriate time. However, this study does not support such an hypothesis. After 300 days of chilling, the percent hatch of *L. aestiva* eggs after warming declined. After more than 400 days of chilling, hatching has not increased again though many of the eggs look viable. Thus this study indicates that eggs cannot survive more than 300 days of constant exposure to 5°C in the laboratory. However, this life span may be quite different in the field, where eggs probably experience long periods of anoxia and exposure to hydrogen sulfide. Although the effect of such parameters on the viability of *L. aestiva* eggs is not known, it has been reported (Uye *et al.,* 1984) that exposure to organic pollution reduces the viability of resting eggs of neritic marine copepods.

From studies of insects and freshwater copepods, I suggested (Marcus, 1979) that *L. aestiva* eggs terminate diapause at different times during the winter in the field, and are held at a stage of pre-hatch readiness because water temperatures are below the threshold for post-diapause development and hatching. This study supports that hypothesis, although the duration of diapause under field conditions appears to differ from that observed in the laboratory where temperature was held constant at 5°C. The data (Marcus, 1984) for eggs collected from bottom sediments in Buzzards Bay, Massachusetts, indicated that the refractory phase was not completed by all eggs in

December. It was suggested that the eggs which failed to hatch at this time were produced later in the fall and had not completed the refractory period. By February all eggs appeared to have completed the refractory phase as evidenced by the high hatch of eggs incubated at 19°C. The present study does not support that suggestion. Because of the seasonal variation in the duration of diapause it is possible that eggs which were collected from sediments in December and did not hatch in the laboratory upon warming, were produced in September as well as in December.

Temperature is not the only environmental parameter that affects the transition from diapause to development. Hatching is also affected by light and oxygen concentration (reviewed by Grice and Marcus, 1981). Although the effect of these parameters on the termination of diapause and the onset of post-diapause development in marine copepods has not been clarified, Brewer (1964) reported that exposure to reduced oxygen concentrations was necessary to terminate egg diapause in the freshwater copepod, *Diaptomus stagnalis,* and Watson and Smallman (1971) suggested that photoperiod was a necessary cue for the resumption of development in *Diacyclops navus.* The transition from dormancy to development is mediated by pH in brine shrimp (Busa and Crowe, 1983).

Diapause is an important factor in the synchronization of life cycles (Tauber *et al.,* 1986). Two possible ways in which synchronization can be achieved are synchronizing the termination of diapause and synchronizing the onset of post-diapause development and hatching. Both mechanisms characterize *L. aestiva* in Woods Hole waters. Since diapause eggs are produced over a span of several months, the longer diapause of eggs produced early in the fall ensures that they do not terminate diapause until winter temperatures have declined below the threshold for post-diapause development. Conversely, the shorter diapause of eggs produced late in the fall ensures that diapause will be completed prior to the time when water temperature exceeds the threshold in the spring. Since diapause in the field terminates by February or March in Woods Hole waters (Marcus, 1984) and hatching does not occur until May (Grice and Gibson, 1975) the precise coincidence of diapause termination should be less important than the coincidence of the onset of post-diapause development in the promotion of synchronous hatching. Thus, as long as the refractory phase is completed before the threshold for post-diapause development or hatching is exceeded, synchronization of hatching should still occur. The coincidence of diapause termination among overwintering eggs may be more critical at more southern latitudes where water temperatures at the time of diapause termination are more likely to exceed the threshold and thus individuals would resume development as soon as the refractory period ended. The results also suggest that diapause is important because it promotes the survival of individuals by conferring cold-hardiness. Diapause eggs which complete their refractory period by January (Marcus, 1984), can tolerate exposure to cold winter temperatures. However, subitaneous eggs do not survive extended exposure to such temperatures (Grice, unpub.). I suggest that the variation in diapause duration expressed by *L. aestiva* is an adaptation that promotes synchronization by ensuring that all individuals terminate diapause at approximately the same time, and survival by conferring cold-hardiness.

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